

Molecular phylogeography of *Pteronotus davyi* (Chiroptera: Mormoopidae) in Mexico

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Pteronotus davyi, Davy's naked-backed bat, is a tropical bat whose current distribution in Mexico covers the watersheds of the Pacific and the Gulf coasts of Mexico, converges at the Isthmus of Tehuantepec, and continues into the Yucatan peninsula. We evaluated phylogeographic relationships among 18 populations in Mexico using data from the nucleotide sequence of the hypervariable II domain of the mitochondrial DNA control region from 105 specimens. Extant populations were distributed over 3 geographic regions (Pacific Coast, Gulf Coast, and Southeastern) that were delineated a priori according to floristic characteristics and biogeography. Coalescent simulations supported a phylogeographic model of 2 refugia situated in the south of Mexico (Arc and Soconusco refuges) during the late Pleistocene, followed by expansion into Pacific Coast, Gulf Coast, and Southeastern groups. The populations of the Southeast were genetically divergent from the Pacific and Gulf Coast populations, supporting the existence of 2 distinct lineages of *P. davyi* in Mexico, likely due to the Isthmus of Tehuantepec acting as a geographical barrier. Examination of our data revealed genetic differentiation of the Pacific and Gulf coastal groups but at a lower level relative to the Southeast. This pattern suggests that the Sierra Madre Occidental, Sierra Madre Oriental, and Sierra Madre del Sur act as physical barriers to dispersal for *P. davyi*. DOI: 10.1644/08-MAMM-A-212R3.1.

Key words: mitochondrial DNA, phylogeography, Pleistocene, population structure, *Pteronotus davyi*

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Pteronotus davyi (Gray, 1838), Davy's naked-backed bat, is an insectivorous bat in the family Mormoopidae, found in Central and South America. It is distinguished from other mormoopids by its small size (forearm < 50 mm) and by having the wing membrane joined over its back, which gives the appearance of a bare back (Adams 1989). Three subspecies are recognized (Adams 1989; Smith 1972): *P. d. davyi*, *P. d. incae*, and *P. d. fulvus*. In Mexico only *P. d. fulvus* has been recorded. It is distributed in 2 separate narrow strips that extend along the Pacific coast (Sonora to Chiapas) and along the Gulf of Mexico (Tamaulipas to Tabasco); the 2 strips converge in the lowlands of the Isthmus of Tehuantepec and continue southeast into the Yucatan peninsula (Adams 1989).

The distribution pattern of *P. davyi* is common among other tropical Mexican bats (Medellín et al. 1997) and is generally explained by ecological requirements (Hall 1981). However, phytogeographic findings (Gómez-Pompa 1973; León-Cazares

and Gómez-Pompa 1970) suggest that during the coldest periods of the Pleistocene the tropical lowlands were displaced southward from their prior distribution in Mexico, and during warmer periods they expanded northward along coastal regions. This phenomenon is expected to have caused numerous episodes of vicariance (Sullivan et al. 2000) associated with successive contractions and expansions in the distribution of tropical lowland fauna (Braun 1988; Hidalgo-Mihart et al. 2004; Russell 1968). Thus, areas on the Pacific and Gulf coasts of Mexico may have acted as separate Pleistocene refugia (Toledo 1982; Wendt 1989). Isolation between coastal regions likely persists due to the interior mountain systems of the Sierra Madre Occidental, Sierra Madre Oriental, and Sierra Madre del Sur. Previous



studies have shown that these mountain ranges act as geographic dispersal barriers for diverse plant and vertebrate species (Fa and Morales 1993; Rzedowski 1978). This is likely true of *P. davyi*.

The occurrence of *P. davyi* is documented from sea level to 1,500 m, but most of the recorded localities are below 600 m (Jiménez-Guzmán and Ceballos 2005), suggesting that mountain peaks up to 3,000 m may act as physical barriers to the species. The Isthmus of Tehuantepec also may act as an east–west or west–east barrier (Graham 1993; Toledo 1982). The isthmus is a transition zone between the Nearctic and Neotropical faunal regions (Escalante et al. 2004; Morrone and Márquez 2001), but the reason it acts as a barrier is unknown.

Few phylogeographic studies have dealt with the native bats of Mexico (Vázquez-Domínguez and Vega 2006), and no such studies examining *P. davyi* have been reported. Although the phylogenetic relationships of *P. davyi* to other taxonomic groups have been inferred (Lewis-Oritt et al. 2001; Simmons and Conway 2001; Van Den Bussche et al. 2002), evolutionary processes at the intraspecific level have yet to be examined.

This work is the 1st effort to evaluate the phylogeography of *P. davyi* throughout its distribution in Mexico and is based on sequences of the hypervariable II (HVII) domain of the mitochondrial DNA (mtDNA) control region. Specifically, we sought to infer the phylogenetic and genealogic relationships among populations by analyzing geographic (population and regional) genetic structure and examining the geographic association among haplotypes.

MATERIALS AND METHODS

Samples and study area.—Tissue samples for this study were collected during a project on echolocation in which animals were captured, measured, biopsied, and released. *Pteronotus davyi* is abundant (not endangered [Secretaría de Medio Ambiente y Recursos Naturales 2002] and Least Concern [International Union for Conservation of Nature and Natural Resources 2008]) and easy to identify in the field. Animal handling and sample collection were conducted in accordance with the guidelines of the American Society of Mammalogists (Gannon et al. 2007).

From a total sample of approximately 440 individual bats (20 each from 22 localities), 105 specimens were used in the present study. This sampling scheme included 18 populations distributed throughout the species' range in Mexico (Fig. 1; Appendix I). Specimens were grouped according to a priori divisions in the range: the Pacific region ($n = 45$), the Gulf of Mexico region ($n = 29$), and the Southeastern region ($n = 31$). These divisions were based on the floristic provinces described by Rzedowski (1978) and included the mountain systems (Sierra Madre Occidental, Sierra Madre Oriental, and Sierra Madre del Sur, and the Trans-Mexican Volcanic Belt; Fig. 1) that separate coastal populations. The Isthmus of Tehuantepec also has been considered a geographical barrier,

because it separates the northern coastal populations from the Southeastern population.

Tissue collection, DNA extraction, amplification, and sequencing.—Biopsy samples were obtained from wing membranes using a 3-mm biopsy punch (Fray Products Corp., Buffalo, New York) and preserved in 70% ethanol. Biopsies that were not used in this study were deposited by RL-W at the Laboratory of Biology and Ecology of Mammals at the Universidad Autónoma Metropolitana Unidad Iztapalapa.

Total DNA was extracted with a DNAeasy Tissue Kit according to instructions of the manufacturer (QIAGEN, Inc., Valencia, California). We amplified the complete HVII domain of the mtDNA control region via polymerase chain reaction (Saiki et al. 1998), using the primers L16517 and HSC (Fumagalli et al. 1996). Primer HSC includes modifications for this study (5'-TGT TTT AGG GGT TTG GCA G-3'). The reactions were carried out in a total volume of 25 μ l with approximately 50 ng of template DNA, 10 μ M of each primer, 200 μ M of each deoxynucleoside triphosphate, 2 mM of $MgCl_2$, and 2.5 units of Taq DNA polymerase (QIAGEN, Inc.). Polymerase chain reaction cycles were as follows: 45 s denaturation at 94°C; 35 cycles of denaturation at 94°C for 30 s, annealing at 62°C for 30 s, and extension at 72°C for 70 s; final extension of 4 min at 72°C. The amplified polymerase chain reaction products were purified using the QIAquick kit (QIAGEN, Inc.) and used in sequencing reactions with the Big Dye Terminator Kit (Perkin-Elmer, Norwalk, Connecticut). Sequencing reactions were electrophoresed in an ABI 3100 automatic sequencer (Applied Biosystems, Foster City, California). Sequence alignment was conducted with the Clustal W algorithm in the Sequence Navigator 1.0.1 program (Applied Biosystems) using default values for all alignment parameters and adjusted by eye.

Phylogenetic and genealogical analyses.—Phylogenetic analysis was performed by maximum-likelihood in PAUP* 4.0b10 (Swofford 2002). The best-fit substitution model of sequence evolution was identified with Modeltest 3.06 program (Posada and Crandall 1998). The Akaike information criterion (AIC—Akaike 1974) recognized the transitional model + invariable sites + gamma shape model (TIM+I+G—Rodríguez et al. 1990) as optimal ($-\ln L = 1,495$, AIC = 3,006.5300), with the following base frequencies: A = 0.26, C = 0.31, G = 0.23, and T = 0.20. The proportion of invariant sites (I) was 0.8090 and the gamma distribution shape parameter (G) was 0.8037. The maximum-likelihood topology was obtained by a 10,000-step quartet puzzling method (Strimmer and Vonhaeseler 1996). A Bayesian analysis also was conducted to assess phylogenetic relationships using the optimal model of evolution determined with Modeltest and the Markov chain Monte Carlo technique in MrBayes 3.0 (Huelsenbeck and Ronquist 2001) with the following parameters: Nst = 6, rates = gamma, Ngen = 2,000,000, frequency = 100, chains = 5. After eliminating the first 2,000 trees as “burn-in,” we constructed a majority-rule consensus tree with Bayesian posterior probabilities for each node.

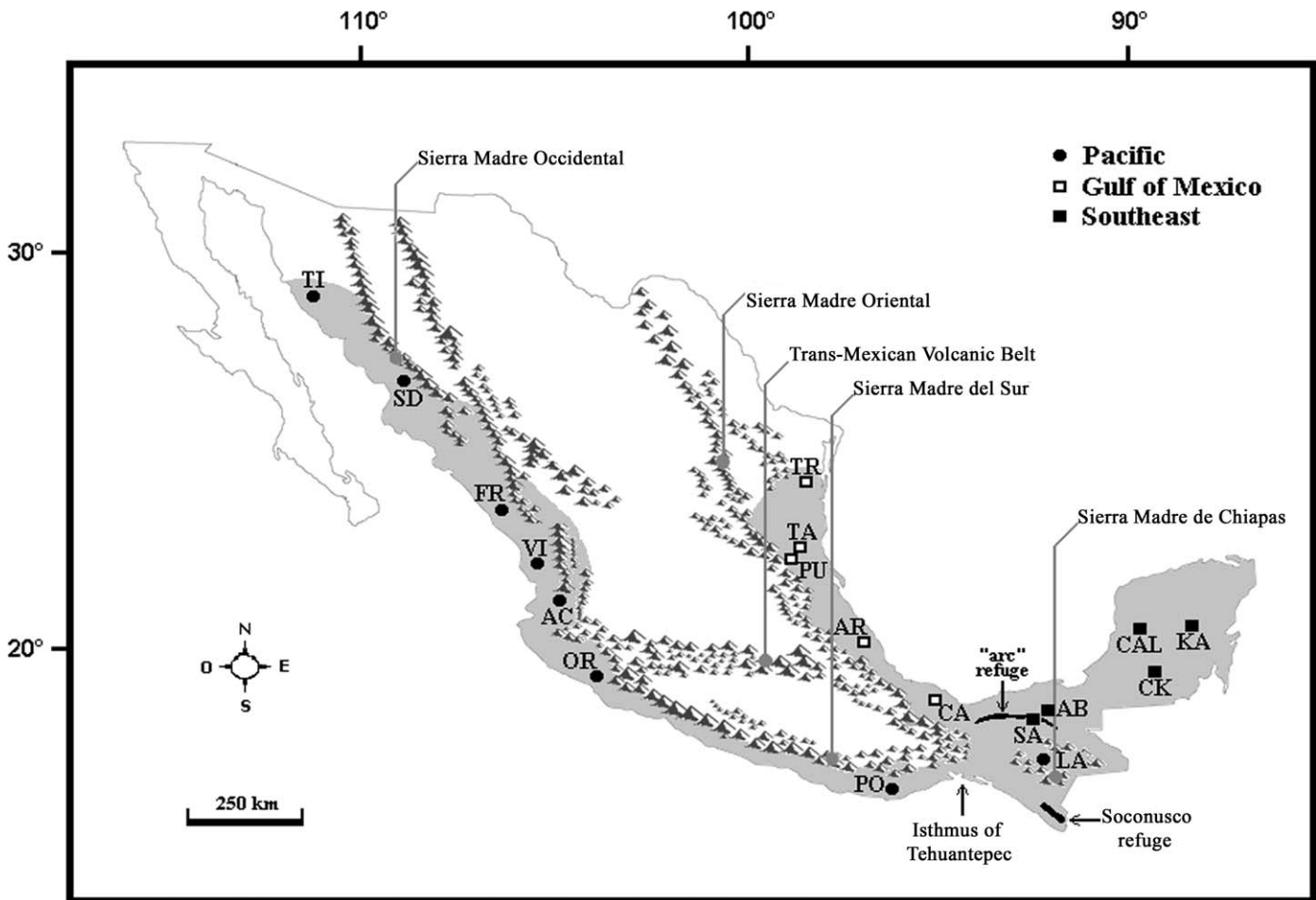


FIG. 1.—Geographical distribution of *Pteronotus davyi* in Mexico in gray (modified from Hall 1981). Closed circles show locations of the Pacific region; closed squares of the Southeastern region; and open squares of the Gulf of Mexico region. The 18 populations sampled are: TI = Tigre; SD = Santo Domingo; FR = Frontera; VI = Viejas; AC = Amatlán de Cañas; OR = Orteses; PO = Pochutla; LA = Laguitos; CA = Catemaco; AR = Arroyo del Bellaco; PU = Pujal; TA = Taninul; TR = Troncones; SA = Sardina; AB = Agua Blanca; CK = Calakmul; KA = Kantemó; CAL = Calcehtok. Important orographic features of Mexico and possible Pleistocene refuges are shown. Arrows indicate the best-supported biogeographic scenario (by all the present analyses).

To establish haplotype relationships, a haplotypic network was constructed based on statistical parsimony (Templeton et al. 1992; Templeton and Sing 1993) using TCS 1.21 software (Clement et al. 2000). This program allows the user to estimate phylogenetic relationships when there are low levels of divergence and provides a 95% plausible set for all haplotype connections.

Phylogeographic and genetic structure analyses.—Average genetic distances among regional groups were evaluated using the Tamura–Nei substitution model (TrN—Tamura and Nei 1993) implemented in MEGA 2.1 (Kumar et al. 2001). We used this model because it has been shown in simulations that if the number of nucleotide substitutions is low (<20%), most models of evolution will render similar genetic distances (Nei and Kumar 2000), and because it is suitable for comparisons of closely related taxa (e.g., Palma et al. 2005).

Isolation by distance was assessed using the Mantel test (Mantel 1967) to determine associations between genetic distances from the TrN model and geographic distances calculated from a 1:3,500,000 scale geographic map of

Mexico. Mantel tests were implemented by the program Mantel 2 (Liedloff 1999) with 1,000 permutations.

The existence of hierarchical genetic structure in *P. davyi* throughout its distribution was assessed by an analysis of molecular variance (AMOVA—Excoffier et al. 1992) with genetic divergence values (F_{ST}), using Arlequin 2.0 (Schneider et al. 2000). Three levels of analyses were conducted: among all populations; Gulf and Pacific Coast versus Southeast; and Gulf Coast versus Pacific Coast. In the latter 2 analyses, variance levels were determined between groups, among populations within groups, and within populations.

Polymorphism levels were estimated by haplotype diversity (h) and nucleotide diversity (π), which were determined for each population and between geographic regions with DNAsp 3.14 (Rozas and Rozas 1999). Using the same program, gene flow (Nm) among geographic regions was calculated as described by Hudson et al. (1992).

The hypothesis of population expansion was tested using the following neutrality tests in combination: Tajima's D (Tajima 1989), Fu's F (Fu 1997), and F^* and D^* of Fu and Li

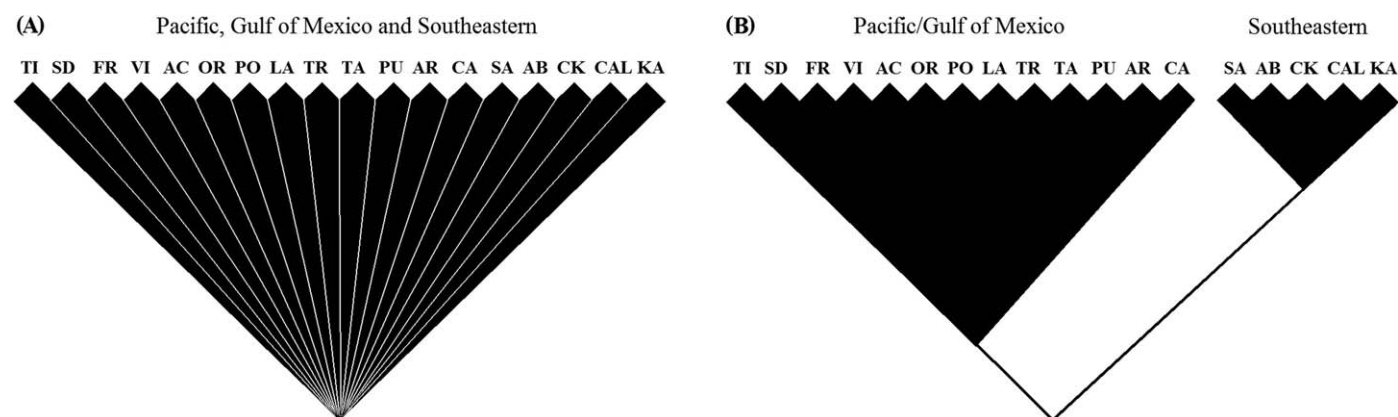


FIG. 2.—Characterization of the population trees representing different models used in the coalescent simulations: A) single-refuge hypothesis, and B) dual-refugia hypothesis. See Fig. 1 for sampling location designations.

(1993). A negative D indicates population expansion. If F is significant but F^* and D^* are not, then a history of population growth is indicated. Population expansion also was tested using R_2 of Ramos-Onsins and Rozas (2002) and the expansion coefficient (S/d) of von Haeseler et al. (1996). The latter is the ratio of the number of variable sites (S) to the average number of pairwise nucleotide differences (d), as implemented in DNAsP. The R_2 statistic (Ramos-Onsins and Rozas 2002) is based on the difference between the number of singleton mutations and the average number of pairwise nucleotide differences. Population growth is indicated by larger expansion coefficients, and population stationarity is indicated by smaller expansion coefficients (von Haeseler et al. 1996). The existence of population expansions was investigated with the frequency distribution of pairwise differences between control region sequences (mismatch distribution—Rogers and Harpending 1992); this analysis was performed in Arlequin 2.0 (Schneider et al. 2000). The null hypothesis of an expanding population (Rogers and Harpending 1992) produces a unimodal distribution, and a ragged distribution indicates a population in stable equilibrium. Finally, correlation analyses between latitude and nucleotide diversities were conducted in order to evaluate possible post-Pleistocene events.

Testing phylogeographic hypotheses using coalescent simulations.—We used Mesquite 1.03 (Maddison and Maddison 2007) to conduct coalescent simulations. Our approach to these simulations was modified from previous works (Anthony et al. 2007; Knowles and Maddison 2002; Russell et al. 2005; Spellman and Klicka 2006). We conducted simulations under 2 a priori hypotheses of Pleistocene population structure, using the maximum-likelihood tree for control region sequences (see the phylogenetic and genealogical analyses section): a single-refugium scenario that posits that the Pacific, Gulf, and Southeastern populations of *P. davyi* were founded from a single ancestral population (Fig. 2A); and a dual-refugia scenario in which populations of *P. davyi* were founded from 2 ancestral populations on either coast near the Isthmus of Tehuantepec as proposed by Toledo (1982) and Wendt (1989; Fig. 2B).

The Isolation with Migration algorithm (IM—Hey 2006) was used to estimate an effective population size of $N_e = 500$ for the species. For *P. davyi*, 1 generation is equal to 2 years (Smith 1972). The branch lengths were expressed as a function of effective population size ($0.02N_e$ —T1, $0.05N_e$ —T2, $0.1N_e$ —T3, $0.6N_e$ —T4) and of generation time x (2×10^3). Extreme values correspond to divergence times dating from the Wisconsin glacial maximum at $\sim 20,000$ years ago (T1) to the mid-Pleistocene interglacial period at $\sim 610,000$ years ago (T4—DeChaine and Martin 2006). Two arbitrary times were tested for intermediate periods (T2 $\sim 50,000$ and T3 $\sim 100,000$).

Mesquite was used to simulate 10,000 coalescent genealogies constrained by the population history predicted by each hypothesis being tested. Each hypothesis was tested with the S statistic of Slatkin and Maddison (1989). This parameter treats populations as categorical variables and measures the minimum number of sorting (or migration) events implied by the genealogy. For the observed genealogy constrained to fit either the 1-refuge or dual-refugia model, the observed S -value was calculated and compared to the distribution of S -values from the coalescent simulations. Comparison of the observed versus null distribution of S -values was used to determine whether the observed genealogy could have been generated under a given model. If the S -value of the reconstructed gene tree fell in the lower 5% tail of the distribution of S -values from simulated gene trees, the null model was rejected.

RESULTS

Composition of nucleotide sequences and haplotypes.—A 555-base pair fragment that covered the complete HVII domain of the mtDNA control region of *P. davyi* was sequenced from 105 specimens of *P. davyi*. This fragment contained 56 variable positions. Sixty-seven distinct haplotypes were identified, with the most common being present in 8.6% of tested individuals (haplotype 4). Ten other haplotypes (14.9%) were present in >1 individual (e.g., haplotype 53, $n = 7$; haplotype 63, $n = 7$; and haplotype

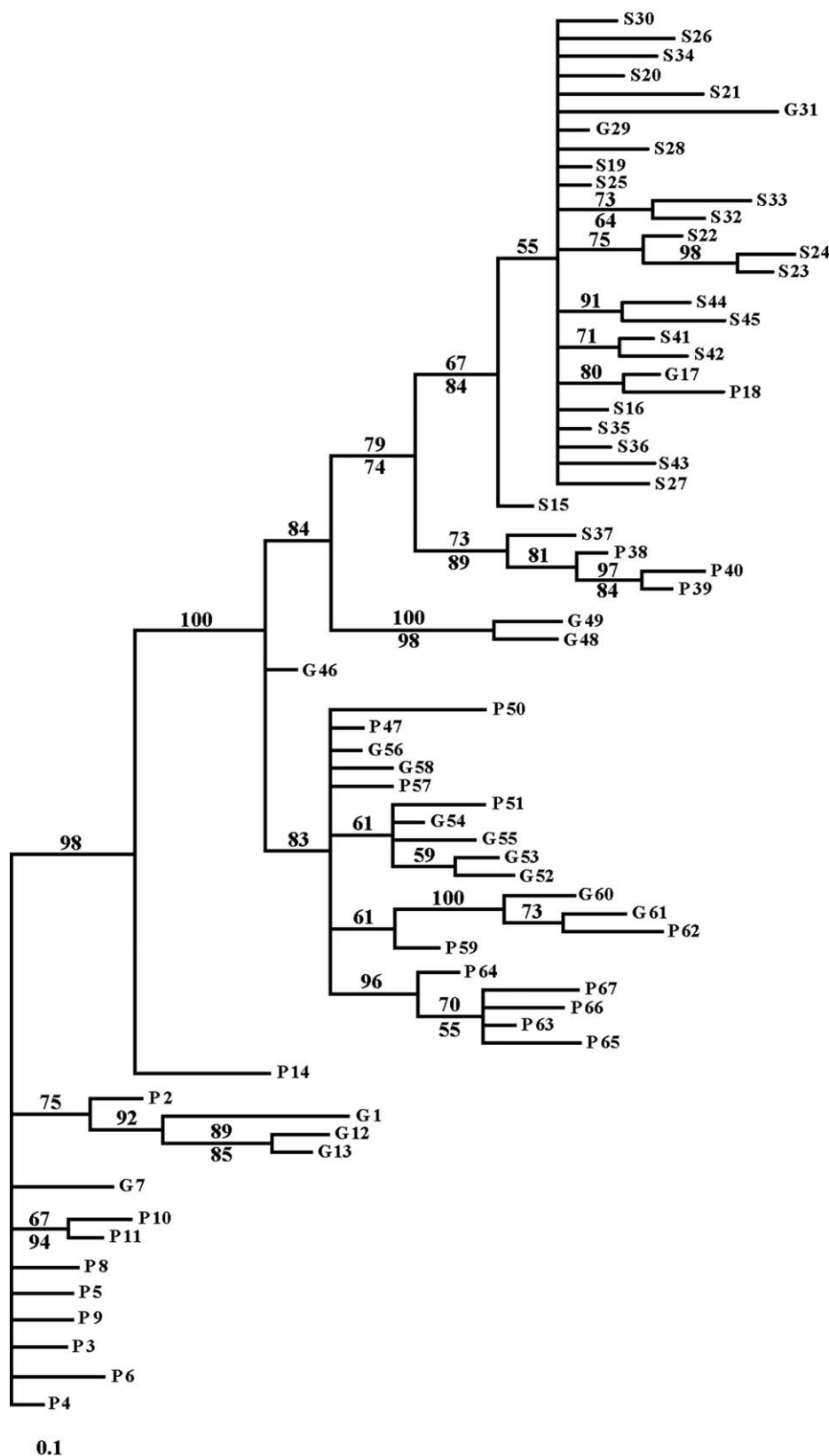


FIG. 3.—Consensus phylogram reached by maximum-likelihood of 67 *Pteronotus davyi* haplotypes using the TIM+I+G model. This topology is consistent with the Bayesian analysis. Below the branches are the quartet puzzling bootstrap values, and above the branches are the Bayesian a posteriori probabilities ($\times 100$). Haplotypes are indicated by a letter (S = Southeastern, P = Pacific, and G = Gulf of Mexico) that indicates the corresponding geographic region, followed by their respective numbers. Bars on the right indicate the geographical distributions of the haplotypes (black = Southeastern; gray = Pacific–Gulf of Mexico).

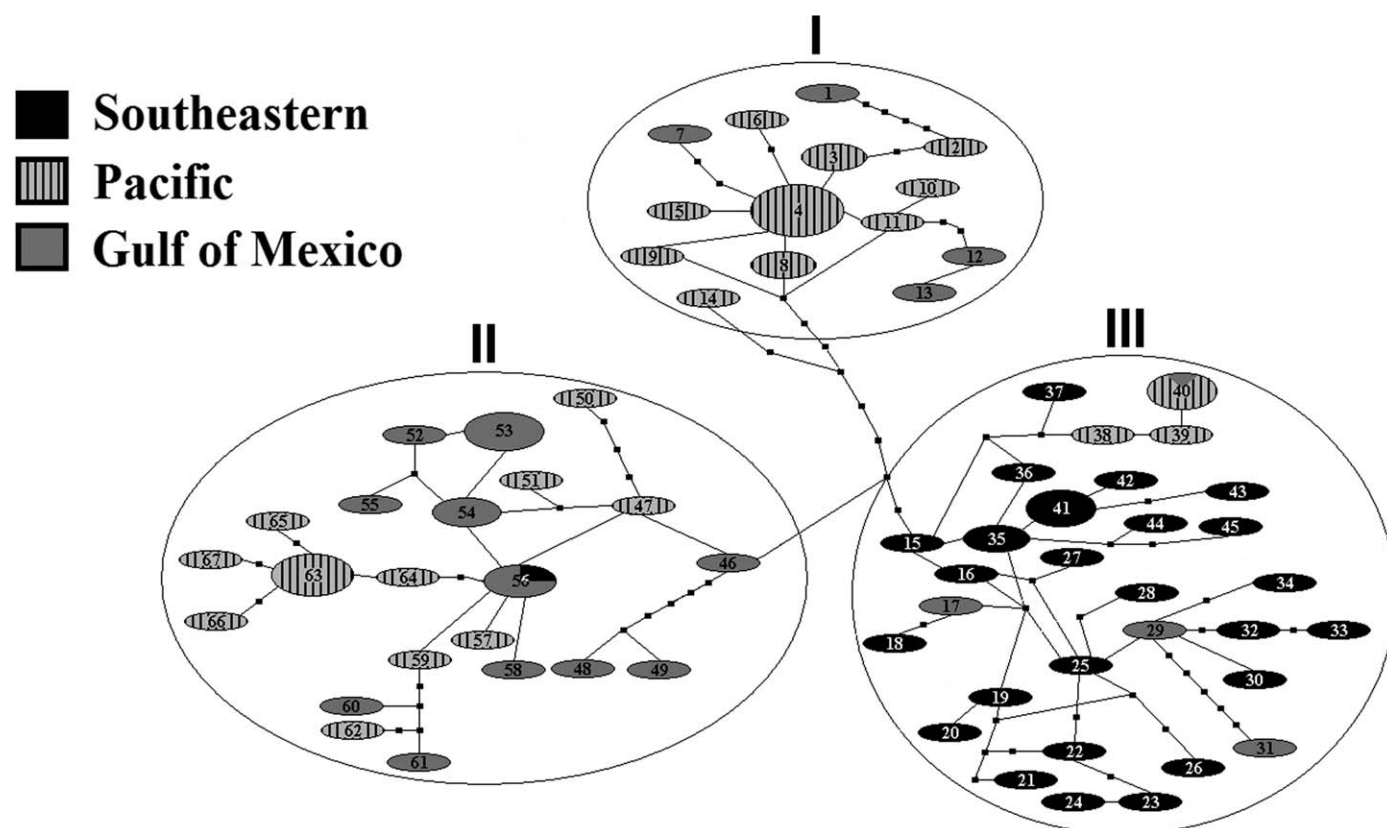


FIG. 4.—Statistical parsimony (TCS) network for control region haplotypes of *Pteronotus davyi*. Haplotypes are represented by numbers within the ovals (1–67). Size of the ovals represents the relative frequency of the indicated haplotype (smallest ovals = 1 individual; large ovals = 9 individuals). Black = Southeastern region; hatched gray = Pacific region; gray = Gulf of Mexico region. Each line connecting an oval or black square (hypothetical internode) indicates 1 base-pair substitution, and each substitution is indicated for each step.

41, $n = 5$). Each haplotype was submitted to GenBank (accession numbers EF989018–EF989084 for haplotypes 1–67, respectively).

Phylogenetic and genealogical analyses.—Similar topologies were generated by maximum-likelihood and Bayesian analyses using the TIM+I+G model. The topologies indicated a near-complete isolation of the Southeastern populations relative to the Pacific and Gulf Coast populations, with moderate support in bootstrap and posterior probability values. The relationship of the Pacific and Gulf populations was less clear in that they did not resolve as separate clades (Fig. 3). The haplotype network resolves 3 groups (Fig. 4) congruent with those on the phylogenetic tree.

Genetic and phylogeographic structure analyses.—Average genetic distance between groups determined using the TrN model was highest between populations of the Pacific–Gulf versus Southeastern (2.05%) and lower between the Pacific and Gulf Coast populations (1.77%). Mantel test results indicated a significant correlation between genetic and geographic distances among the 18 populations (Mantel coefficient: $z = 7,409.75$, $P < 0.05$), suggesting isolation by distance. The same pattern was observed for Pacific and Gulf Coast regions only ($z = 3,601.93$, $P < 0.05$), and among populations of the Gulf Coast region only ($z = 110.79$, $P < 0.05$). When analyzed separately, populations within the Pacific Coast region ($z = 964.35$, $P > 0.05$) and

within the Southeastern region ($z = 61.84$, $P > 0.05$) did not show isolation by distance.

Results of AMOVA indicated that the highest proportion of the genetic variance was within populations (Table 1), but high interregional variance also was detected between Pacific–Gulf Coast and Southeastern populations, and between Pacific and Gulf Coast regions. Regional differentiation values (F_{ST}) were high and significant for Pacific–Gulf Coast versus Southeastern ($F_{ST} = 0.42$, $P < 0.05$), and for Pacific versus Gulf Coast regions ($F_{ST} = 0.23$, $P < 0.05$).

The 3 geographical regions contained similar levels of haplotype diversity (Table 2). Nucleotide diversity was low in the Southeastern region compared to Pacific and Gulf Coast regions. Within each region populations showed high haplotypic diversity. The π values varied widely (Table 2). In the Gulf region populations inhabiting Catemaco and Arroyo del Bellaco (Veracruz) had the highest π values. Gene flow, as estimated by an index of the number of migrants (N_m) between the Pacific–Gulf and Southeastern regions, was low ($N_m = 0.86$), but higher between the Pacific and Gulf regions ($N_m = 2.96$).

A history of population growth or range expansion was indicated strongly for all regions. The tests of F and R_2 were significant, but the D -test of Tajima and F^* - and D^* -tests of Fu and Li were not. The regions also were characterized by high values of the expansion coefficient (Table 3). The

TABLE 1.—Analysis of molecular variance results for populations of *Pteronotus davyi* from the Gulf of Mexico, Pacific, and Southeastern regions. AP = among populations, WP = within populations, AG = among groups, APWG = among populations within groups.

Grouping	Source of variation	Sum of squares	Variance components	Percentage of variation	F_{ST}
General	AP	188.6	1.3	30.1	0.301*
	WP	275.2	3.1	69.9	
Gulf-Pacific versus Southeastern	AG	85.8	1.8	32.7	0.429*
	APWG	102.9	0.6	10.2	
Gulf versus Pacific	WP	275.2	3.2	57.1	
	AG	26.5	0.6	12.5	0.231*
	APWG	68.1	0.5	10.6	
	WP	213.1	3.5	76.9	

* $P < 0.05$.

mismatch distribution was unimodal (Fig. 5A), indicating a recent range expansion. The Southeastern region also showed a unimodal distribution. The Pacific and Gulf Coast curves were not strictly unimodal, although they were consistent with recent population growth (Figs. 5B and 5C). A tendency toward decreased nucleotide diversity with increasing latitude was found in both the Gulf Coast and Pacific regions ($y = -0.0039x + 0.0914$, $r^2 = 0.898$ and $y = -0.0004x + 0.0236$, $r^2 = 0.713$, respectively).

Based on the observed sequences, we calculated $S = 3$ and $S = 17$ (Slatkin and Maddison 1989) for the single-refugium and dual-refugia hypotheses, respectively. For all values of

TABLE 2.—Molecular diversity indices for the studied populations and regions. Sample size (n), number of haplotypes (k), haplotypic or Nei's diversity (h), and nucleotide diversity (π).

Population	n	k	h	π
Pacific region	45	25	0.933	0.0149
El Tigre, Sonora	7	3	0.524	0.0101
Santo Domingo, Sonora	7	5	0.857	0.0116
La Frontera, Sinaloa	7	4	0.714	0.0115
Las Viejas, Nayarit	7	6	0.952	0.0157
Amatlán de Cañas, Nayarit	5	4	0.900	0.0155
Los Ortices, Colima	6	6	1.000	0.0156
Pochutla, Oaxaca ^a	1	1	—	—
Los Laguitos, Chiapas	5	4	0.900	0.0147
Gulf region	29	19	0.933	0.0135
Catemaco, Veracruz	7	7	1.000	0.0194
Arroyo del Bellaco, Veracruz	7	7	1.000	0.0191
El Pujal, San Luis Potosí	5	3	0.700	0.0079
Taninul, San Luis Potosí	5	4	0.900	0.0028
Los Troncones, Tamaulipas	5	3	0.800	0.0018
Southeastern region	31	25	0.972	0.0084
La Sardina, Tabasco	7	7	1.000	0.0097
Agua Blanca, Tabasco	5	4	0.900	0.0115
Calakmul, Campeche	7	7	1.000	0.0066
Kantemó, Quintana Roo	5	5	1.000	0.0068
Calcehtok, Campeche	7	7	1.000	0.0085
Total	105	67	0.979	0.0172

^a The Pochutla (Oaxaca) population was not included in the genetic diversity analysis because only 1 individual was sampled.

TABLE 3.—Demographic analyses for *Pteronotus davyi* (includes the expected results under the scenario of population expansion).

	General	Pacific	Gulf	Southeastern	Expansion
Tajima's D	-0.538	-0.233	-1.085	-1.496	Not significant
Fu's F	-53.408*	-6.257*	-4.268*	-20.031*	Significant
Fu and Li's F^*	-1.021	-1.191	-0.833	-2.042	Not significant
Fu and Li's D^*	-1.070	-1.421	-0.532	-1.845	Not significant
Ramos-Onsins and Rozas's R_2	0.078*	0.099*	0.091*	0.0624*	Significant
Expansion coefficient (S/d)	6.177	4.690	5.287	6.825	High

* $P < 0.05$.

divergence times ($0.6N_e$, $0.1N_e$, $0.05N_e$, and $0.02N_e$) used in Mesquite simulations, the hypothesis of 1 refuge between the Wisconsin glacial and mid-Pleistocene interglacial periods ($S = 3$, $P > 0.05$) was rejected (data not shown). Examination of our data supports the dual-refugia hypothesis for $0.05N_e$ ($S = 17$, $P < 0.05$; Fig. 6B), indicating a model of Pleistocene separation for the Pacific/Gulf Coast versus Southeastern regions of approximately 50,000 years (25,000 generations). Other values of divergence times used for the dual-refugia model were rejected (Figs. 6A, 6C, and 6D).

DISCUSSION

Phylogeographic relationships.—The most recent hypothesis regarding the distribution of Mormoopidae suggested that *P. davyi* originated and diversified in the northern Neotropics (Mexico and the Caribbean) before entering South America (Dávalos 2006). The current distribution pattern of *P. davyi* is best explained by its ecological requirement of low tropical forest (Hall 1981). Examination of phytogeographic data (Gómez-Pompa 1973; León-Cazares and Gómez-Pompa 1970) indicates that during cold periods of the Pleistocene, low tropical forest in Mexico was displaced to the south more than once. During warming periods, low tropical forest expanded north via corridors, as documented for other plants and animals (Delgadillo 1987; Halffter 1976; Schmidly 1977).

Our results suggest a role for Pleistocene refugia in structuring genetic diversity of *P. davyi* and support the biogeographic hypothesis of contraction followed by coastal expansion of lowland tropical forests in Mexico. Although the coalescent simulations in the present study suggest that the Gulf Coast, Pacific Coast, and Southeastern regions were colonized from Pleistocene dual-refugia (Fig. 6B), which split 25,000 generations ago ($\sim 50,000$ years) during the Wisconsin glaciation, coalescent simulations provide no information regarding the putative location of these refugia. However, some traditional analyses provide an alternative means of inferring the location of a refugium (Avise 2000). For instance, the mismatch distribution for all localities had a single peak (Fig. 5A). This suggests that populations expanded from a centralized location and genealogically are connected tightly (Ibrahim et al. 1996). The diversity statistics and neutrality tests supported this inference (Tables 2 and 3).

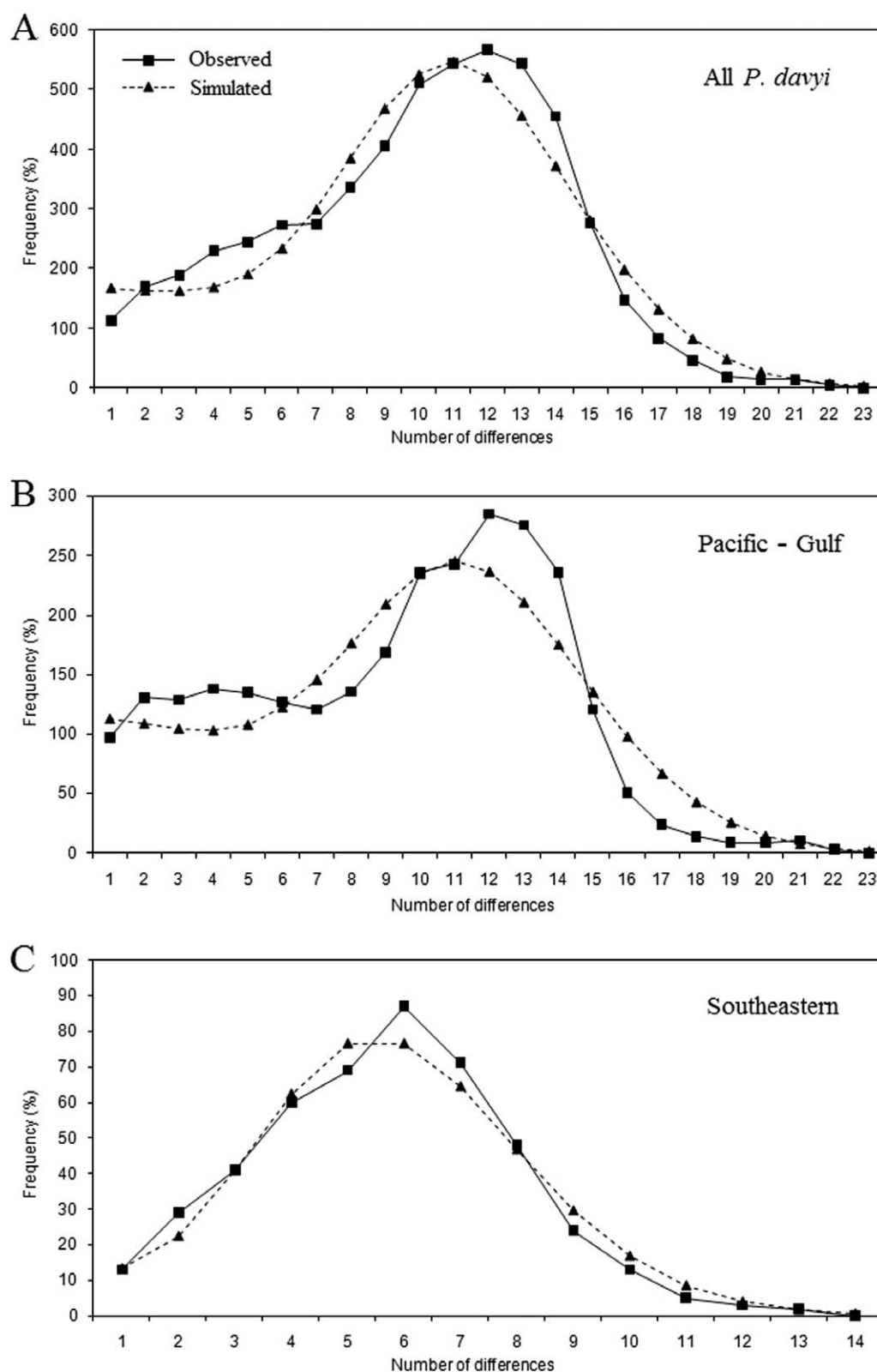


FIG. 5.—Mismatch distribution of pairwise nucleotide differences among control region sequences of *Pteronotus davyi*: A) Overall, B) Pacific–Gulf, and C) Southeastern. Solid lines indicate expected distribution under sudden population expansion model (Rogers and Harpending 1992).

Furthermore, mismatch distributions for Pacific–Gulf Coast and Southeastern regions (Figs. 5B and 5C), and examination of nucleotide diversity data, suggest that *P. davyi* may have experienced expansion and occupation of the Pacific, Gulf

Coast, and Southeastern regions from 2 refuges located near the Isthmus of Tehuantepec.

Some potential refuges in the South of Mexico during the cold Pleistocene periods have been proposed: Conroy et al.

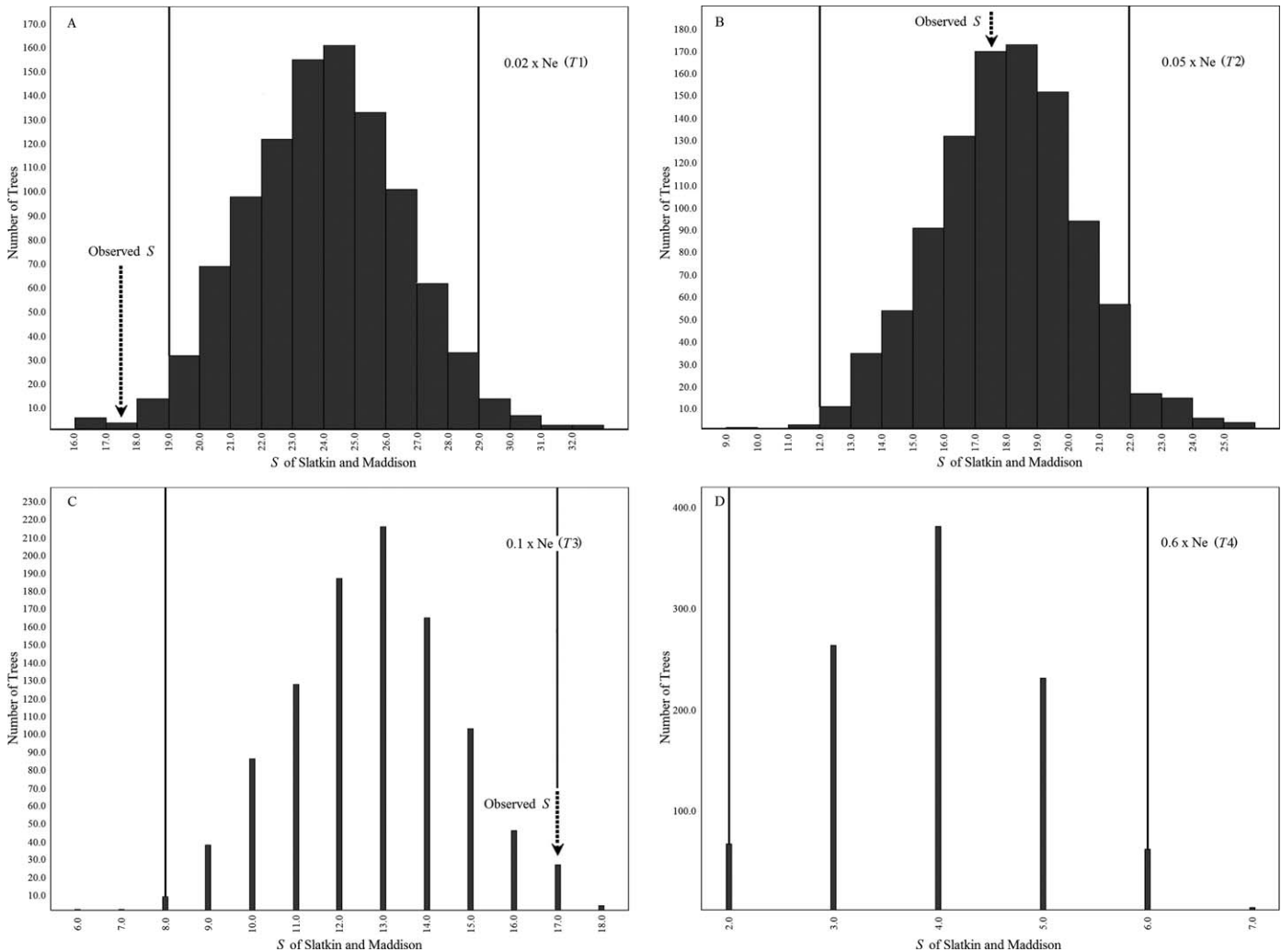


FIG. 6.—Distribution of gene flow statistics (Slatkin and Maddison 1989) for 10,000 simulated gene trees within 4 population trees. Population trees differ in their branch lengths, expressed as a function of the effective population size (N_e). A) Branch lengths = $0.02N_e$. B) Branch lengths = $0.05N_e$. C) Branch lengths = $0.1N_e$. D) Branch lengths = $0.6N_e$. Observed $S = 17$ (indicated by the dashed line). Bold black lines represent the 95% confidence interval for the distribution.

(2001) suggested the mountains in southeastern Mexico; Wendt (1989) proposed the Uxpanapa zone in the Isthmus of Tehuantepec and the general “arc” that extends from the southeastern extremes of Veracruz to Tabasco and Chiapas (Fig. 1); and Toledo (1982) suggested the Lacandona and Soconusco regions in Chiapas. It is possible that northern populations of *P. davyi* found refuge in Uxpanapa and Chiapas zones and then expanded to their current distribution at the end of the Pleistocene.

It appears that animals from the 2 refuges expanded to colonize the Southeastern region and the 2 coastal regions (Pacific and Gulf). This hypothesis is supported by multiple lines of evidence: the phylogenetic topologies; the high average TrN distance (2.05%) and F_{ST} -value (0.42, $P < 0.05$) between the Southeastern and Pacific–Gulf Coast regions; and the existence of only 1 common haplotype between them (haplotype 56). This pattern of geographic subdivision is consistent with what has been observed in other terrestrial mammalian taxa from the region. For example, Sullivan et al.

(2000) found subspecific divergence in *Reithrodontomys sumichrasti* based on the separation of the trans-Isthmian populations from those in central and southeastern Mexico (5.6–7.7% using uncorrected sequence divergence). Sullivan et al. (1997) reported uncorrected sequence divergence values of 6.9–8.9% in the *Peromyscus aztecus*–*P. hylocetes* complex along the same geographical feature.

The Southeastern and Pacific–Gulf population groups of *P. davyi* also show a trend of separation in the trans-Isthmian region, as revealed by the phylogenetic and genealogical results. Similar geographic patterns have been reported between subspecies of the bats *Leptonycteris curasoae* (Wilkinson and Fleming 1996) and *Pteronotus parnellii* (Hall 1981). Considering this scenario, 2 possible taxonomic units of *P. davyi* exist in Mexico: 1 that extends into the Yucatan peninsula and the Isthmus of Tehuantepec and another that stretches from the Pacific Coast, including Sonora and Tamaulipas to Oaxaca, and along the Gulf Coast to Veracruz.

Genetic structuring: Pacific versus Gulf.—The Pacific and Gulf regions showed less structure between coasts than that observed between both coasts combined and the Southeastern region. They also showed lower divergence values and high levels of gene flow, suggesting that they had a common Pleistocene origin. The neutrality tests indicated that these populations later underwent expansion along each coastal slope. Inland mountain ranges with elevations above 3,000 m could act as contemporary barriers to dispersal between the Pacific and Gulf regions, given that most populations of *P. davyi* live between sea level and 600 m (Jiménez-Guzmán and Ceballos 2005). Thus, the higher similarity between east–west populations reflects a recent, shared past.

Although we observed only 1 shared haplotype (haplotype 40) between the 2 coastal regions (Pacific and Gulf), the possibility of gene flow occurring at lower altitudes on the Isthmus of Tehuantepec cannot be ruled out. This could explain the structuring of the haplotype network, particularly groups I and II, which contained haplotypes from both regions (Fig. 4). This hypothesis also is supported by the resolved phylogeny, where the Pacific–Gulf Coast clade showed haplotypes from both regions.

Intraregional structure.—The observed tendency toward decreased nucleotide diversity with increasing latitude in both the Gulf Coast and Pacific regions is a geographic pattern consistent with a postglacial stepping stone model of expansion toward the north along corridors of tropical lowland forests on both coasts. Decreased nucleotide diversity along the leading edge of a postglacial expansion front has been observed in plants (González-Astorga et al. 2006) and other bat species (Ruedi and Castella 2003) from Mexico. Erosion of genetic diversity also could be enhanced by the isolation of some populations due to habitat fragmentation (González-Astorga and Castillo-Campos 2004), a phenomenon that could be particularly acute in the Gulf coast given the current rapid decline of northern tropical forests. This scenario would explain the low levels of nucleotide diversity observed in the populations of San Luis Potosí and Tamaulipas (Pujal, Taninul, and Troncones; Table 2) and the presence of isolation by distance in this region. Although populations in the Southeastern region did not show isolation by distance or exhibit a latitude-related diversity pattern, it appears that the Yucatan peninsula populations have lower diversity than those on the Isthmus of Tehuantepec, suggesting that they too are part of a recent distributional expansion (Table 2).

Our analyses indicated that populations of *P. davyi* are not panmictic but rather are structured and separated by geographic barriers according to models of isolation and colonization. We suggest that this occurred mainly during the Wisconsin portion of the Pleistocene. More than 15 bats species (Medellín et al. 1997) and other mammals (Hall 1981) have a distribution pattern similar to that of *P. davyi*. This peculiar pattern has traditionally been attributed to ecological characteristics. However, it also can be attributed to complex historical processes. Examination of these data reveals the need to gain a perspective on the population structure of *P. davyi*

outside of Mexico, and the need for phylogeographic studies of closely related species with similar geographic patterns (e.g., *Pteronotus parnellii* or *P. personatus*) to elucidate aspects of coevolution, comparative phylogeography, or both.

RESUMEN

Pteronotus davyi es un murciélago tropical que en México presenta un área de distribución que abarca las vertientes del Pacífico y del Golfo de México con unión en el Istmo de Tehuantepec, hasta la Península de Yucatán. Evaluamos las relaciones filogeográficas a nivel intraespecífico, utilizando datos de secuencias nucleotídicas del dominio hipervariable HVII de la región control del ADN mitocondrial en 105 especímenes de 18 poblaciones en México, las cuales se dividieron a priori en 3 regiones geográficas (Pacífico, Golfo y Sureste) de acuerdo a sus características florísticas y biogeográficas. Las simulaciones coalescentes apoyan un modelo filogeográfico de 2 refugios en el sur de México (refugio del Arco y del Soconusco), a partir de los cuales tuvo lugar una expansión durante el Pleistoceno hacia las 3 regiones geográficas, seguido por una división que dio origen a la separación de los grupos Pacífico–Golfo y Sureste. Reconocemos una separación genética de las poblaciones de la región Sureste con respecto a las poblaciones de las regiones Pacífico y Golfo, apoyando la posibilidad de 2 distintos linajes de *P. davyi* en México, promovido por la presencia del Istmo de Tehuantepec como barrera geográfica. Identificamos una estructura genética diferenciada entre las 2 vertientes costeras (Pacífico y Golfo) aunque a un nivel inferior respecto a la región Sureña, detectando una profunda influencia de los sistemas montañosos Sierra Madre Occidental, Sierra Madre Oriental y Sierra Madre del Sur como barreras físicas para la dispersión de *P. davyi*.

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APPENDIX I

Specimens examined.—Collection localities, GenBank accession numbers, and the corresponding haplotype are provided. The relative frequency of the haplotypes per locality is indicated in square brackets.

Pteronotus davyi (67 haplotypes).—El Tigre, Sonora, 29°34.52'N, 110°49.19'W (EF989080-hapl63 [5]; EF989021-hapl4 [1]; EF989057-hapl40 [1]); Santo Domingo, Sonora, 27°02.64'N, 109°00.69'W (EF989021-hapl4 [3]; EF989057-hapl40 [1]; EF989026-hapl9 [1]; EF989027-hapl10 [1]; EF989056-hapl39 [1]); La Frontera, Sinaloa, 23°25.18'N, 105°54.61'W (EF989021-hapl4 [4]; EF989020-hapl3 [1]; EF989057-hapl40 [1]; EF989081-hapl64 [1]); Las Viejas, Nayarit, 21°51.40'N, 105°08.81'W (EF989080-

hapl63 [2]; EF989021-hapl4 [1]; EF989022-hapl5 [1]; EF989057-hapl40 [1]; EF989083-hapl66 [1]; EF989084-hapl67 [1]); Amatlán de Cañas, Nayarit, 20°48.46'N, 104°23.54'W (EF989025-hapl8 [2]; EF989023-hapl6 [1]; EF989064-hapl47 [1]; EF989082-hapl65 [1]); Los Ortices, Colima, 19°04.81'N, 103°43.59'W (EF989020-hapl3 [1]; EF989028-hapl11 [1]; EF989031-hapl14 [1]; EF989055-hapl38 [1]; EF989067-hapl50 [1]; EF989068-hapl51 [1]); Pochutla, Oaxaca, 15°48.35'N, 96°29.00'W (EF989079-hapl62 [1]); Los Laguitos, Chiapas, 16°49.31'N, 93°08.51'W (EF989076-hapl59 [2]; EF989019-hapl2 [1]; EF989035-hapl18 [1]; EF989074-hapl57 [1]); Catemaco, Veracruz, 18°27.71'N, 95°11.18'W (EF989024-hapl7 [1]; EF989029-hapl12 [1]; EF989046-hapl29 [1]; EF989048-hapl31 [1]; EF989063-hapl46 [1]; EF989065-hapl48 [1]; EF989078-hapl61 [1]); Arroyo del Bellaco, Veracruz, 19°13.32'N, 96°38.34'W (EF989018-hapl1 [1]; EF989030-hapl13 [1]; EF989034-hapl17 [1]; EF989049-hapl66 [1]; EF989073-hapl56 [1]; EF989075-hapl58 [1]; EF989077-hapl60 [1]); El Pujal, San Luis Potosí, 21°51.60'N, 98°56.30'W (EF989070-hapl53 [3]; EF989057-hapl40 [1]; EF989073-hapl56 [1]);

Taninul, San Luis Potosí, 21°56.11'N, 98°53.16'W (EF989070-hapl53 [2]; EF989069-hapl52 [1]; EF989071-hapl54 [1]; EF989072-hapl55 [1]); Los Troncones, Tamaulipas, 23°46.20'N, 99°12.17'W (EF989070-hapl53 [2]; EF989071-hapl54 [2]; EF989073-hapl56 [1]); La Sardina, Tabasco, 17°27.50'N, 92°46.40'W (EF989036-hapl19 [1]; EF989045-hapl28 [1]; EF989038-hapl21 [1]; EF989050-hapl33 [1]; EF989052-hapl35 [1]; EF989054-hapl37 [1]; EF989058-hapl41 [1]); Agua Blanca, Tabasco, 17°37.20'N, 92°28.34'W (EF989058-hapl41 [2]; EF989040-hapl23 [1]; EF989041-hapl24 [1]; EF989073-hapl56 [1]); Calakmul, Campeche, 18°31.37'N, 89°49.42'W (EF989033-hapl16 [1]; EF989037-hapl20 [1]; EF989049-hapl32 [1]; EF989051-hapl34 [1]; EF989052-hapl35 [1]; EF989053-hapl36 [1]; EF989058-hapl41 [1]); Kantemó, Quintana Roo, 19°55.84'N, 88°47.46'W (EF989059-hapl42 [1]; EF989061-hapl44 [1]; EF989032-hapl15 [1]; EF989042-hapl25 [1]; EF989044-hapl27 [1]); Calcehtok, Campeche, 19°59.42'N, 89°45.83'W (EF989060-hapl43 [1]; EF989062-hapl45 [1]; EF989039-hapl22 [1]; EF989043-hapl26 [1]; EF989052-hapl35 [1]; EF989058-hapl41 [1]; EF989047-hapl30 [1]).